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Diurnal patterns of photosynthesis, chlorophyll fluorescence, and PRI to evaluate water stress in the invasive species, *Elaeagnus umbellata* Thunb.

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Abstract Photosynthesis, chlorophyll fluorescence, and hyperspectral reflectance were used to evaluate diurnal changes of *Elaeagnus umbellata* to quantify physiological responses of the invasive species during times of stress. Field measurements showed that *E. umbellata* is able to maintain higher levels of photosynthesis relative to nearby *Quercus alba* plants, with less water loss. Plants subjected to progressive drought were able to recover photosynthesis one day following re-watering. Laboratory and field measurements revealed decreasing $\Delta F/F_m$ values in response to drought stress, with little corresponding decrease in photochemical reflectance index values. This research supports the view that xanthophyll cycle dissipation is not the photoprotective mechanism at work for *Elaeagnus* species under water stress. *Elaeagnus umbellata* maintains photosynthetic carbon assimilation even under drought conditions, in part, due to chemical dissipation of excess light, and in part because of morphological features that limit excess radiation while maximizing photosynthetic carbon gain. These characteristics may contribute to the invasive success of *E. umbellata*.

Keywords *Elaeagnus* · PRI · Fluorescence · Photosynthesis · Chlorophyll · Drought stress

Introduction

A host of theories exists to explain the mechanisms of biological invasions, as well as the negative consequences to the invaded communities (Hufbauer and Torchin 2007). Much of the current literature is concerned with competitive exclusion of native species, although few studied invasions lead to native exclusion across a community (Bruno et al. 2005); therefore, arrival and establishment of an exotic depend upon resource availability in the presence of natives. Following arrival and exploitation of resources, if an environment provides the opportunity for the invading plant to increase its density, growth rate, dispersal success, or reproductive rate, the alien may expand aggressively at the expense of the local community (Hufbauer and Torchin 2007). Physiological and morphological traits of plants may help explain invasive success in a particular system as they are related to growth and resource utilization (Baruch and Goldstein 1999). Higher photosynthetic rates reflect the potential of a species to accumulate more biomass, thus depleting local resources and likely shading out native competitors. Research focused on understanding the mechanisms with which invasive species are able to succeed could potentially help prevent future invasions (Baruch and Goldstein 1999).

Elaeagnus umbellata Thunb. (Elaeagnaceae) is a deciduous, drought resistant invasive woody species that forms extensive, dense thickets. Commonly, it is a large multiple-stem shrub, rarely treelike with a single trunk, reaching a maximum height of five to six meters. Leaves are elliptic with entire margins, having sparse stellate trichomes on adaxial surfaces, and a dense covering of peltate trichomes on abaxial surfaces. *Elaeagnus umbellata* is native to coarse-textured, moderately or well-drained soils of Asia (Ahmad et al. 2006), but was introduced to

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North America as an ornamental shrub and has spread from cultivation in the mid- and eastern United States (Ebinger and Lehnert 1981). While many introduced species tend to establish and thrive in edge areas and fragmented forests (Benítez-Malvido and Martínez-Ramos 2002), *E. umbellata* also succeeds in the understory of pre-existing forests (Yates et al. 2004). In addition, it readily colonizes disturbed areas or poor-quality soil where its association with *Frankia*, a nitrogen-fixing endosymbiont, may confer a unique advantage over other local species (Yates et al. 2004). *Elaeagnus umbellata* produces large quantities of small fleshy drupes, which are consumed by birds and may be dispersed in great numbers over large distances (Suthers et al. 2000; Ahmad et al. 2006). As a result, *E. umbellata* is often found to be rapidly expanding into nonnative habitat (Yates et al. 2004).

Many studies have reported on the invasiveness of *Elaeagnus* species in various systems and have discussed the threat to ecological functioning of those systems once well established (Nestlerode et al. 1987; Katz and Shafroth 2003; Yates et al. 2004), but research investigating the physiology of the species, especially in areas where it is considered invasive, remains limited (Gong et al. 2006; Zhao et al. 2007). Research into physiological responses during times of stress may facilitate the understanding of how species are able to successfully invade new systems. The photosynthetic efficiency of many plants decreases under conditions of stress (Chaves et al. 2002) and many mechanisms have evolved in response including photorespiration, detoxification of photosynthetically produced reactive molecules and the water–water cycle (Demmig-Adams and Adams 1992; Flexas and Medrano 2002; Apel and Hirt 2004). Despite the many pathways for excess energy dissipation, a large body of literature supports the role of xanthophyll-mediated photoprotection under conditions of natural stress, especially water stress (Demmig-Adams and Adams 1996; Flexas et al. 2002; Winkel et al. 2002; Evain et al. 2004). Changes in the de-epoxidation state of carotenoid pigments of the xanthophyll cycle, and subsequent accumulation of zeaxanthin are reflected by absorbance changes centered near 531–535 nm and indirectly measured via the Photochemical Reflectance Index (PRI; Bilger et al. 1989; Gamon et al. 1992; Ruban et al. 1993). This study explores the physiological characteristics of *E. umbellata* throughout the growing season and during drought in both the laboratory and field. Our goals were (1) to quantify seasonal and diurnal changes in plant physiological status in field conditions and (2) to quantify changes in physiology under progressive drought conditions and after re-watering in a controlled laboratory experiment. In addition to traditional physiological measurements, we used remote sensing detection to infer xanthophyll cycle

changes as a possible mechanism for excess energy dissipation during times of stress.

Materials and methods

Study site and plant material

The field portion of this study was conducted at Fort A.P. Hill, Virginia (38° 5.602' N, 77° 20.075' W) from May to August 2008 where the expansion of *E. umbellata* has occurred over many years. The climate is temperate with maximum summer temperatures ranging from 28 to 31°C and an average rainfall of 1,131 mm per year. *Elaeagnus umbellata* thickets primarily occur along roadsides at Fort A.P. Hill, although the species is expanding at forest edges and in old fields, and we have observed it in the understory as well. We selected a thicket that was 30 × 290 m and adjacent to an agricultural field. Five individual plants were selected for field measurements and followed throughout the day. For laboratory measurements, saplings of *E. umbellata* were collected from the study area, transplanted into 2-L plastic pots and grown for 3 months in an environmental chamber before experimentation. Saplings were ~40 cm tall during experimentation. Leaves of plants grown in the lab were morphologically similar to adult plants in the field, including leaf size, thickness and with sparse stellate trichomes on adaxial surfaces, and dense peltate trichomes on abaxial surfaces.

Field measurements

Monthly measurements of stomatal conductance, net photosynthesis, leaf fluorescence, leaf xylem pressure potential, and leaf reflectance were collected every 60–90 min on sunny days (900–1,600) on the fourth or fifth fully expanded sunlit leaf of individual *E. umbellata* shrubs ($n = 5$). The same leaves were used for all measurements at a specific time. Leaves were not repetitively measured throughout the day due to destructive sampling for xylem pressure potential measurements. Incident photosynthetic photon flux density (PPFD), stomatal conductance (g_s), leaf net photosynthesis (A_n) and light-adapted measurements of chlorophyll fluorescence were measured using a portable infrared gas analyzer with a pulse amplitude-modulated leaf chamber fluorometer (LI-6400, LI-COR, Inc. Lincoln, NE). The relationship between maximal fluorescence in a light-adapted leaf after a saturating pulse of light (F'_m) and steady-state fluorescence prior to any saturating pulse (F_s) was used to estimate the effective quantum yield of photosystem II:

$$\Delta F/F'_m = [F'_m - F_s]/F'_m$$

Leaf xylem pressure potentials (Ψ) were quantified with a Scholander pressure chamber (Model 650, PMS, Corvallis, OR). Midday measurements of g_s , A_n , and Ψ were made on nearby *Quercus alba* plants from June to August for comparison of physiological measurements to a native species.

Leaf and canopy spectral reflectance (350–2,500 nm) were measured using an ASD FieldSpec Pro Full Range reflectance spectrometer (Analytical Spectral Devices, Inc., Boulder, CO). The ASD spectral resolution is ~ 1 –3 nm from the visible to the short-wave infrared. The fore-optic of the spectrometer was held from a tall pole in a nadir position at a distance ~ 1 m above the canopy using an 8° field-of-view on a cloudless day. To acquire a representative value, multiple spectra were collected and averaged for each canopy. Data were reduced from binary using the manufacturer's software. A NIST spectralon reflectance standard was used as a white reference to optimize instrument gains prior to each canopy measurement. This standard provides a near 100% lambertian reflectance surface for calibration. Using the resulting reflectance values, several canopy reflectance indices were calculated. Concurrent measurements of $\Delta F/F'_m$ were made on 50 leaves at each site with a pulse amplitude-modulated leaf chamber fluorometer (LI-6400, LI-COR, Inc., Lincoln, NE) to represent canopy fluorescence.

Laboratory measurements

Elaeagnus umbellata saplings were grown in a Conviron environmental chamber (CMP 3244, Controlled Environments Limited, Asheville, NC) under a PPFD of approximately $700 \mu\text{mol m}^{-2} \text{s}^{-1}$, 48% relative humidity, a photoperiod of 14 h, and a day/night temperature of 30/25°C. Drought stress was induced by withholding watering from treatment plants for 8 days (at which point all plants had wilted and stomata had closed) followed by a recovery period in which treatment plants received water until soils were saturated (measurements made on days 9 and 11). Responses of drought-treated plants were compared to well-watered control plants.

Plant responses to drought were quantified by measuring stomatal conductance to water vapor (g_s), leaf net photosynthesis (A_n), light-adapted fluorescence ($\Delta F/F'_m$), and plant reflectance using the aforementioned instruments ($n = 5$). Measurements were conducted midday (1,100–1,300 h) on days 1, 4, 6, and 8 for drought experiments, and days 9 and 11 during the recovery. Measurements of xylem pressure potential were made only on days 8, 9, and 11 due to destructive sampling.

Statistical analysis

For field experiments, two-way analysis of variance (ANOVA) was used to test for significant interactions between month and time for stomatal conductance, photosynthesis, chlorophyll fluorescence, xylem pressure potential, and reflectance indices (Zar 1999). Significant differences among months were identified with Tukey post hoc tests ($\alpha = 0.05$). In cases where a significant interaction occurred, one-way ANOVAs were used to test for variations among months at specific times.

Variations in photosynthetic characteristics, stomatal conductance, fluorescence, and reflectance indices relative to control plants over time were analyzed with repeated measures ANOVA for the drought stress experiment (Zar 1999). Day was specified as the repeated factor (within-subject), and treatment as fixed effect (between-subjects). The validity of a within-subjects test depends on sphericity of the data (Von Ende 1993). A measure of deviation that addresses this assumption, the Huynh–Feldt (H–F) correction (Huynh and Feldt 1976), was calculated and adjusted P values (H–F P) were reported. Dunnett's tests ($\alpha = 0.05$) were used to identify significant differences in treatment means from controls for individual days. All statistical analyses performed using SAS 9.1 software (SAS Institute, Inc.).

Results

Field physiological measurements

Air temperatures were averaged throughout the growing season with average maximum highs reaching 30°C in June and July. Although precipitation for May through August 2008 was approximately equal to the 30-year average (398 mm), precipitation for May and June was 56% above the average for those 2 months. Precipitation for July and August was 52% below the average (Fig. 1). Precipitation in August was 78% below the monthly average. Water stress during these months was evidenced by the physiological response of *E. umbellata*. Xylem pressure potentials were significantly lower in July and August ($F = 36.56$, $P < 0.0001$; Fig. 2). Maximum daily values in these months were lower than the lowest values experienced earlier in the season. By August, xylem pressure potential was as low as -2.1 MPa (Fig. 2).

Stomatal conductance also significantly decreased as the season progressed with the lowest daily values experienced in August ($F = 18.24$, $P < 0.0001$; Fig. 2). There was a significant interaction between month and time of day ($F = 6.16$, $P < 0.0001$). Stomata opened earlier in the day during June, with significantly higher rates at 900 and

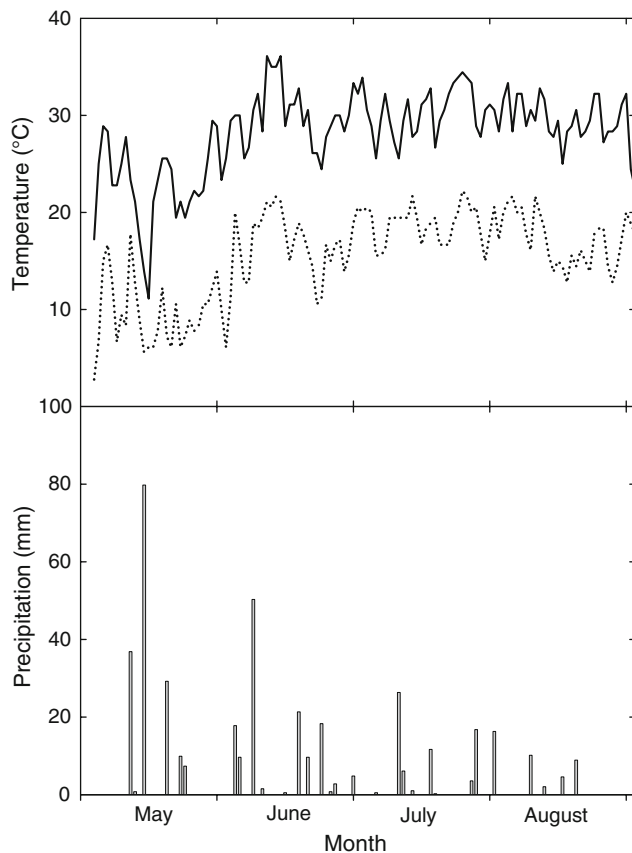


Fig. 1 Variations in maximum (solid line) and minimum air temperature (dotted line) and precipitation as measured from a meteorological station at Fort A.P. Hill from 1 May to 31 August 2008

1,000 ($F = 6.78$, $P = 0.0037$ and $F = 6.96$, $P = 0.0033$, respectively). Photosynthetic rates were highest in June and lowest in both May and August over the course of the day ($F = 67.68$, $P < 0.0001$; Fig. 2). There was a significant interaction between time of day and month ($F = 24.49$, $P < 0.0001$). Analysis of assimilation rates at specific times revealed that photosynthesis was significantly lower in August only at 1100 ($F = 182.31$, $P < 0.0001$) and rates at 1,000 were significantly higher during June and August ($F = 28.12$, $P < 0.0001$).

Monthly comparisons of midday physiological measurements were made on nearby *Q. alba* trees. During June there were no statistical differences between species in stomatal conductance or photosynthesis ($F = 0.01$, $P = 0.9110$; $F = 0.85$, $P = 0.3690$; respectively; Fig. 3). Stomatal conductance values did not differ in July and August for *Q. alba* plants relative to *E. umbellata* ($F = 0.02$, $P = 0.8998$; $F = 3.74$, $P = 0.0869$), but photosynthesis during both months was significantly lower in *Q. alba* (July $F = 38.95$, $P < 0.0001$; August $F = 6.21$, $P = 0.0227$; Fig. 3). Xylem pressure potentials were significantly lower during June and July for *Q. alba* plants

($F = 6.63$, $P = 0.0191$, $F = 36.13$, $P < 0.0001$), but there was no difference in August between species ($F = 3.44$, $P = 0.0799$; Fig. 3).

Daily time courses of incident PPFD ranged from 1,100 to 1,600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ over the course of measurements (Fig. 4). From May to July, $\Delta F/F'_m$ followed the diurnal variations in PPFD, but this pattern was not seen during August (Fig. 4). $\Delta F/F'_m$ was highest in June and lowest in May ($F = 6.90$, $P = 0.0003$; Fig. 4). There was a significant interaction between month and time of day ($F = 4.22$, $P < 0.0001$). Diurnal variations in $\Delta F/F'_m$ were relatively similar during the first 3 months, with higher values occurring during the morning and late afternoon. By August this pattern had dramatically changed. During August, $\Delta F/F'_m$ was significantly lower at 900 ($F = 3.91$, $P = 0.0286$) and significantly higher than the other months at 1230 ($F = 3.5$, $P = 0.04$) and remained high throughout the rest of the afternoon (Fig. 4). Leaf trichome densities ($n = 10$) were higher on the abaxial surface 35 ± 2 relative to the adaxial surface 16 ± 2 .

Values of PRI were highest during August and lowest during May ($F = 16.01$, $P < 0.0001$; Fig. 4). There was a significant interaction between time of day and month ($F = 2.54$, $P = 0.0032$). Diurnal variations in PRI changed with month, with lowest values occurring at different times of the day. PRI reached low values at midday, between the hours of 1230–1400 ($F = 8.93$, $P = 0.0010$, $F = 4.87$, $P = 0.0136$, respectively) with the lowest levels occurring in May and June. Interestingly, PRI values remained high during August throughout the course of the day despite evidence of water stress. There were significant relationships between $\Delta F/F'_m$ and PRI during May ($F = 33.86$, $P < 0.0001$, $r^2 = 0.57$), June ($F = 85.93$, $P < 0.0001$, $r^2 = 0.75$), and July ($F = 28.14$, $P < 0.0001$, $r^2 = 0.55$). In contrast, there was no relationship between the two parameters during August ($F = 1.29$, $P = 0.2654$, $r^2 = 0.04$; Fig. 5).

Laboratory physiological measurements

Significant reductions in physiological parameters of drought-treated plants were observed in *E. umbellata*. Significant day \times treatment interactions were observed in stomatal conductance ($F = 12.49$, $P < 0.0001$) and photosynthesis ($F = 15.45$, $P < 0.0001$) in response to drought. Control and treatment plants differed significantly by day 6 for both physiological parameters (Fig. 6). Visible signs of stress were also observed on day 6 of drought treatment in some *E. umbellata* plants. By day 8, all drought-treated plants showed signs of stress as evidenced by extremely wilted leaves and essentially closed stomata ($14 \pm 5 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$). Additionally, xylem pressure potentials were significantly lower by day 8 in drought-treated plants

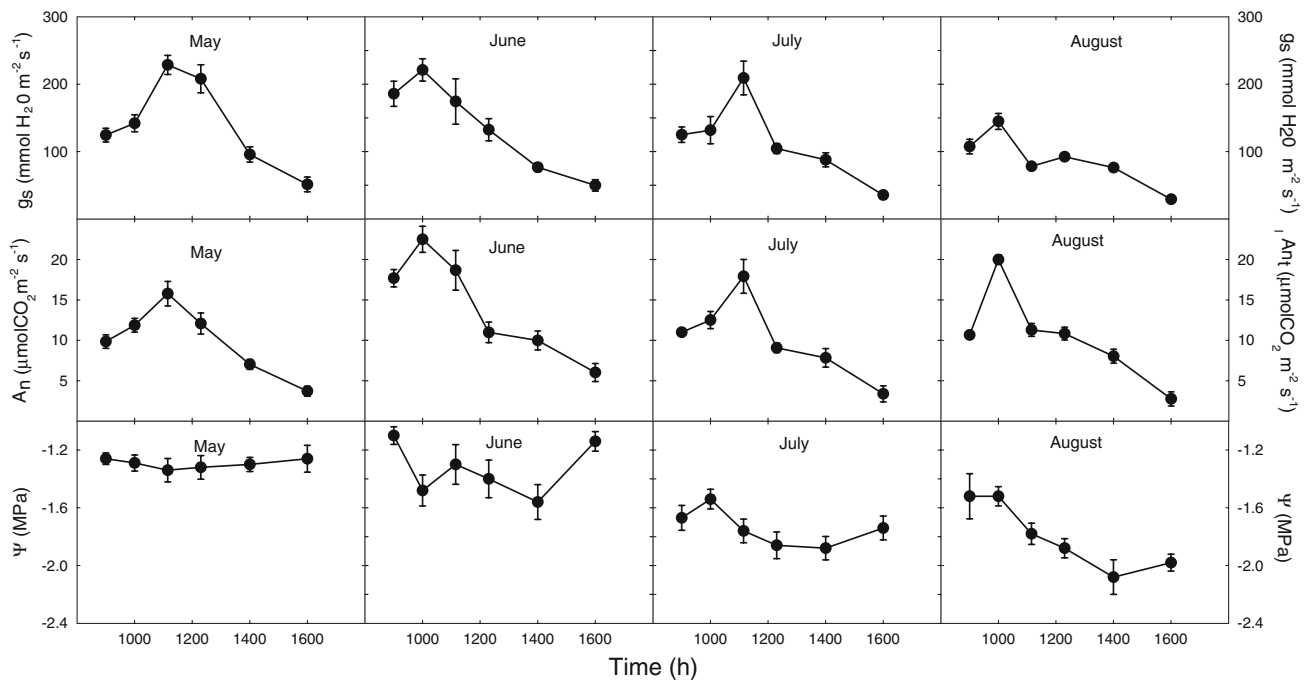


Fig. 2 Monthly variations in stomatal conductance (g_s), net photosynthesis (A_n), and xylem pressure potential (Ψ). Values represent mean \pm 1 standard error

(-3.68 ± 0.53 MPa) relative to controls (-1.26 ± 0.05 MPa; $F = 20.62$, $P = 0.0019$). By day 9 (1 day after recovery), xylem pressure potentials were beginning to recover (-1.68 ± 0.16 MPa), and by day 11 there was no difference between drought and control plants ($F = 1.18$, $P = 0.1097$). A significant day \times treatment interaction for $\Delta F/F'_m$ ($F = 6.83$, $P < 0.0001$) was also seen (Fig. 6). Drought-stressed plants had significantly lower $\Delta F/F'_m$ values by day 6 of the experiment (0.42 ± 0.03) compared to control plants (0.53 ± 0.03), which occurred on the same day as visible signs of stress. By day 8, $\Delta F/F'_m$ values reached 0.35 ± 0.04 . There were no significant changes in PRI between control and drought-treated plants throughout the experiment ($F = 0.26$, $P = 0.9037$), and no significant day \times treatment interaction ($F = 0.41$, $P = 0.8370$; Fig. 6). There was also no significant relationship between PRI and $\Delta F/F'_m$ ($F = 0.10$, $P = 0.75$, $r^2 = 0.001$; Fig. 7).

During the recovery period, stomatal conductance remained significantly lower than control plants; however, photosynthetic rates of drought-treated plants reached control levels by the first day of recovery. $\Delta F/F'_m$ values also recovered to control values by the first day (Fig. 6).

Discussion

Elaeagnus umbellata is a drought-tolerant plant that can maintain some degree of photosynthesis even during times of water stress. During July and August, xylem

pressure potentials reached values lower than -2.0 MPa while remaining photosynthetically active. Stomata opened earlier in the day and reached maximum rates before noon throughout the summer. Similar stomatal conductance responses were observed throughout the course of a day in *E. angustifolia* growing at the edge of the Linze Oasis in the HeXi corridor of northern China (Gong et al. 2006). Maximum net photosynthesis also occurred early in the day, with significantly lower rates in the afternoon. Despite lower stomatal conductance and xylem pressure potentials during August, high photosynthetic rates were still observed in the morning and some level of photosynthesis was maintained throughout the afternoon. Photosynthetic rates measured in our study are as high as those reported in *E. angustifolia* (Gong et al. 2006) and higher than those reported in *E. umbellata* seedlings (Côté et al. 1988). Relative to nearby *Quercus alba* plants, *E. umbellata* was able to reach maximum photosynthetic rates with lower stomatal conductance and higher xylem pressure potentials. The ability to maximize carbon assimilation even during times of water stress confers an obvious advantage to *E. umbellata* and contributes to its overall invasive potential.

The structural morphology of *E. umbellata* allows for survival in semiarid environments (Klich 2000) and may play a role in the enhancement of photosynthesis during times of water stress. Sun-exposed leaves of *E. umbellata* possessed trichomes on both leaf surfaces throughout the growing season, with the abaxial side always more

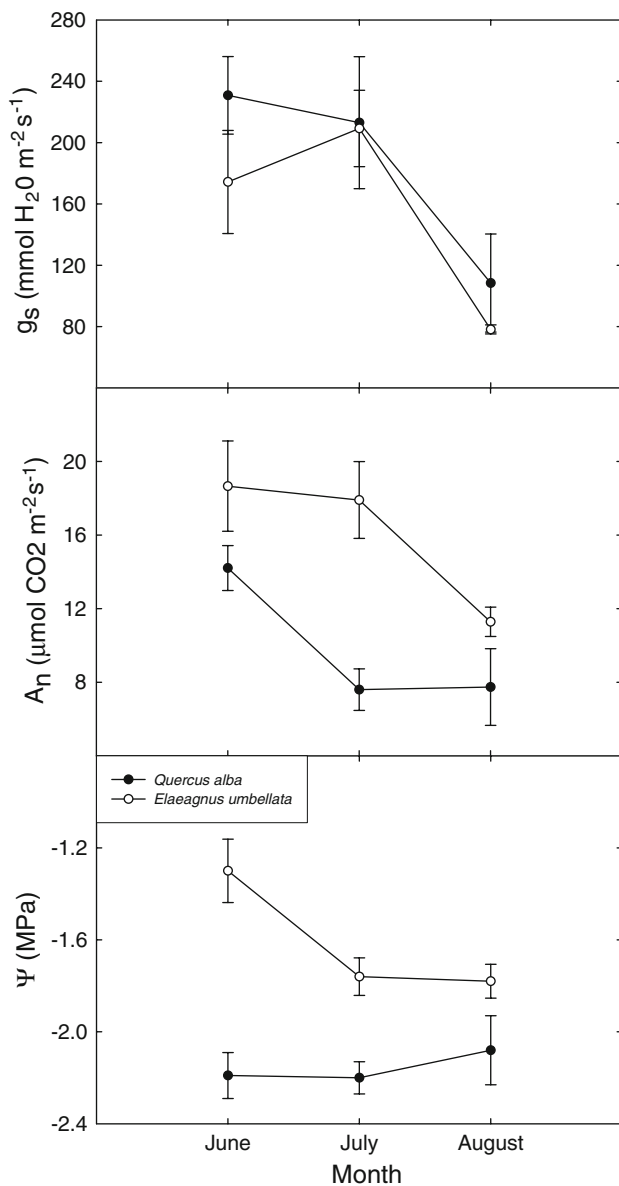


Fig. 3 Midday monthly variations in stomatal conductance (g_s), net photosynthesis (A_n), and xylem pressure potential (Ψ) for *Elaeagnus umbellata* and *Quercus alba* plants. Values represent mean \pm 1 standard error

pubescent. This is often considered to be an adaptive trait in xeric environments (Ehleringer and Björkman 1978) and also characteristic of the related invasive species, *E. angustifolia* (Klich 2000). Trichomes can function to protect leaves during times of stress in multiple ways. Leaf absorptance is reduced by pubescence by enhancing reflectance (Ehleringer et al. 1976), thereby reducing the stress of a high light environment. The presence of trichomes allows plants to avoid lethal leaf temperatures during hot summer months and allows leaves to maintain near optimal temperatures for photosynthesis (Ehleringer and Mooney 1978; Ehleringer 1982).

Interestingly, *E. umbellata* possesses many shade-like characteristics that enhance photosynthesis and light capture throughout the growing season and enable plants to maintain high photosynthetic rates while reducing the light load in a high light environment. Because the abaxial side of a leaf is more pubescent than the adaxial, this dense pubescent layer causes the abaxial surface to be lighter in color, acting as a reflective surface to enhance light capture by reflecting the light back in, while avoiding the effects of high light due to the presence of adaxial trichomes (Klich 2000). Light is also reflected back toward leaves positioned toward the interior of the canopy. Morales et al. (2002) found the presence of trichomes to act as a protective light-filtering mechanism for sun leaves of *Q. ilex* subsp. *Bal-lota*. The structural architecture of *E. umbellata* is very similar to plants in shaded environments. Leaves are positioned facing south and leaf angles are horizontal to maximize sunlight capture (Brantley and Young 2009).

Earlier in the season, $\Delta F/F'_m$ followed expected diurnal changes (Bellot et al. 2004) and PRI tracked changes in $\Delta F/F'_m$ as seen in the significant linear relationships during May, June, and July. By August, when the plants showed physiological signs of water stress in stomatal conductance and xylem pressure potential values, $\Delta F/F'_m$ did not follow diurnal variations in PPFD and there was no relationship between $\Delta F/F'_m$ and PRI. During the drought experiments, physiological parameters were significantly lower in drought-treated plants by day 6 of the experiment, and xylem pressure potentials reached values lower than those experienced in field studies. $\Delta F/F'_m$ also declined by day 6 due to water stress. After re-watering, stomatal conductance values remained lower than controls; however, photosynthesis and $\Delta F/F'_m$ recovered by the next day and were not significantly different from controls for the remainder of the experiment, even though xylem pressure potentials had not reached control levels. This is similar to responses seen in the field during August. PRI did not differ significantly between control and drought-treated plants at any time during the experiment and there was no relationship between PRI and $\Delta F/F'_m$ for drought-treated plants, indicating that xanthophyll pigment changes are not responsible for photoprotection of photosystem II during drought conditions. Zhao et al. (2007) found that *E. angustifolia* plants under water stress did not dissipate excess energy through the increase in the pool size of xanthophyll cycle components, as has been reported for many species (Chaves et al. 2002). Instead, as water stress increased, the reversible inactivation of partial PSII reaction centers played an important role in photoprotection (Zhao et al. 2007). Furthermore, the presence of trichomes has been found to decrease the need for xanthophyll-based photoprotection in the short term (Morales et al. 2002). Our research supports the view that xanthophyll cycle

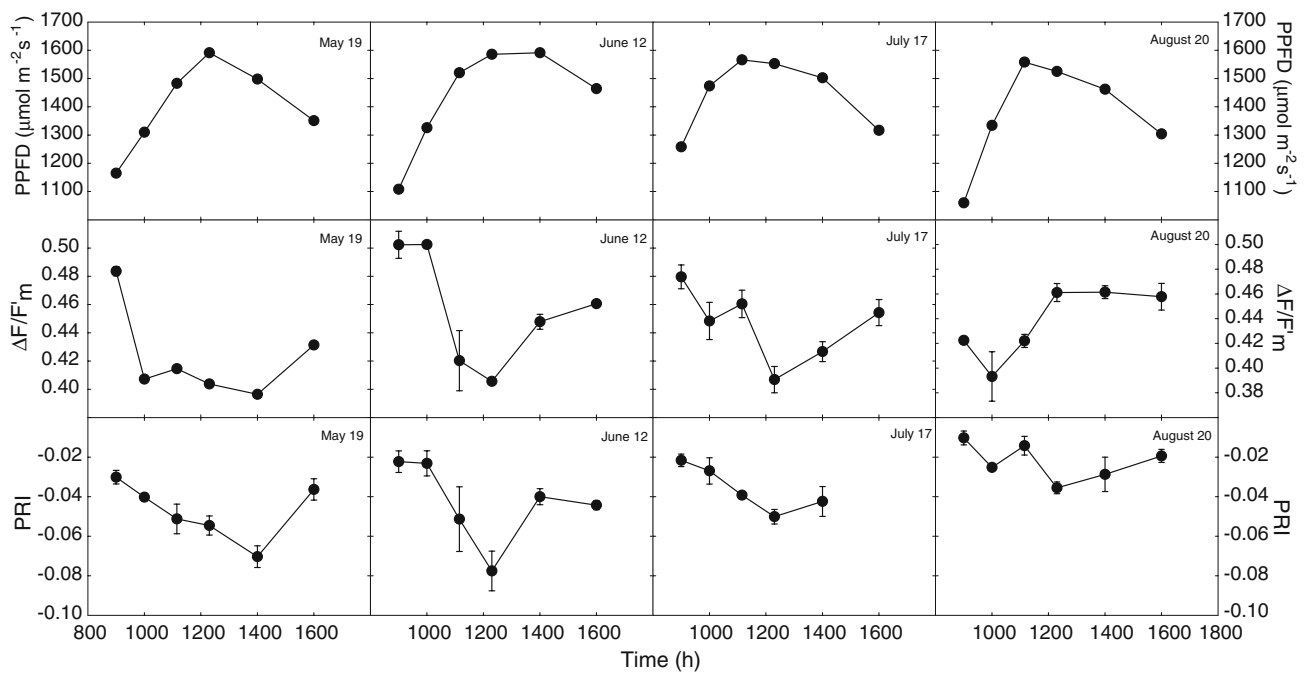


Fig. 4 Monthly variations in incident PPFD, $\Delta F/F'_m$, and PRI. Values represent mean \pm 1 standard error

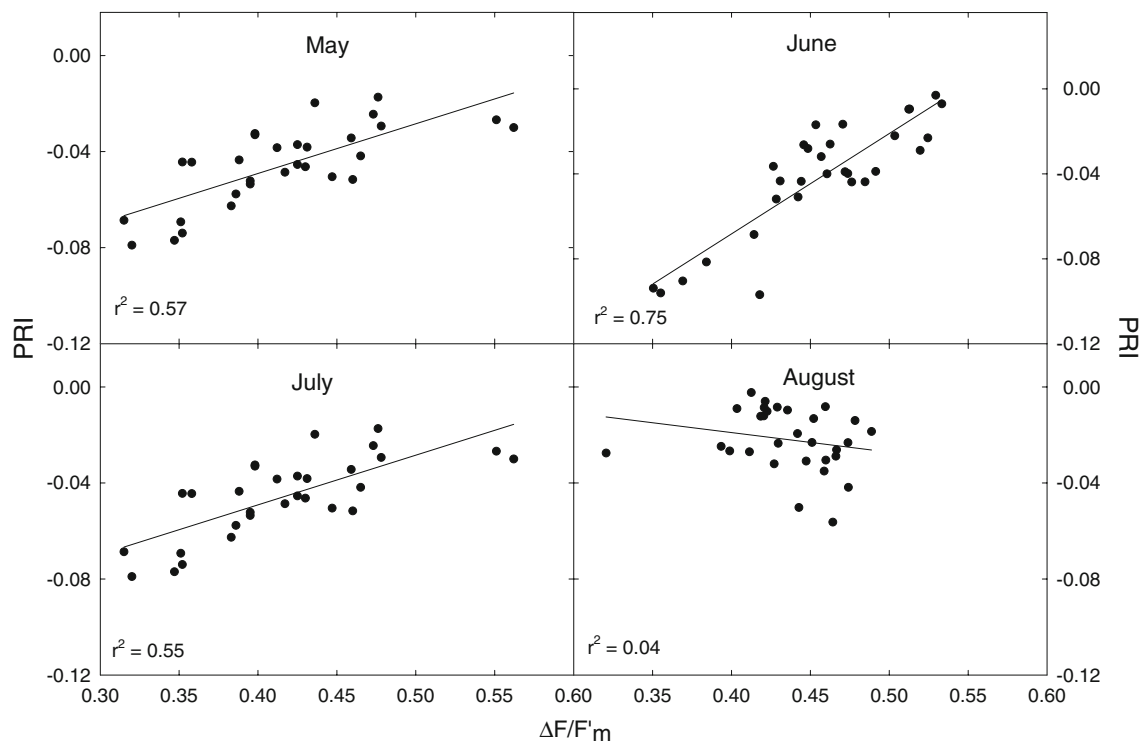


Fig. 5 Monthly relationships between PRI and $\Delta F/F'_m$ from field measurements

dissipation is not the photoprotective mechanism at work for *Elaeagnus* species under water stress.

In summary, *E. umbellata* has many characteristics that enable high photosynthetic rates even during times of stress, determined in both field and lab studies. This

mechanism contributes to the ability to be a successful invader. The presence of trichomes provides a highly reflective surface to avoid excess light energy, while canopy structure is similar to those of shade plants, allowing for maximum light capture. In laboratory studies,

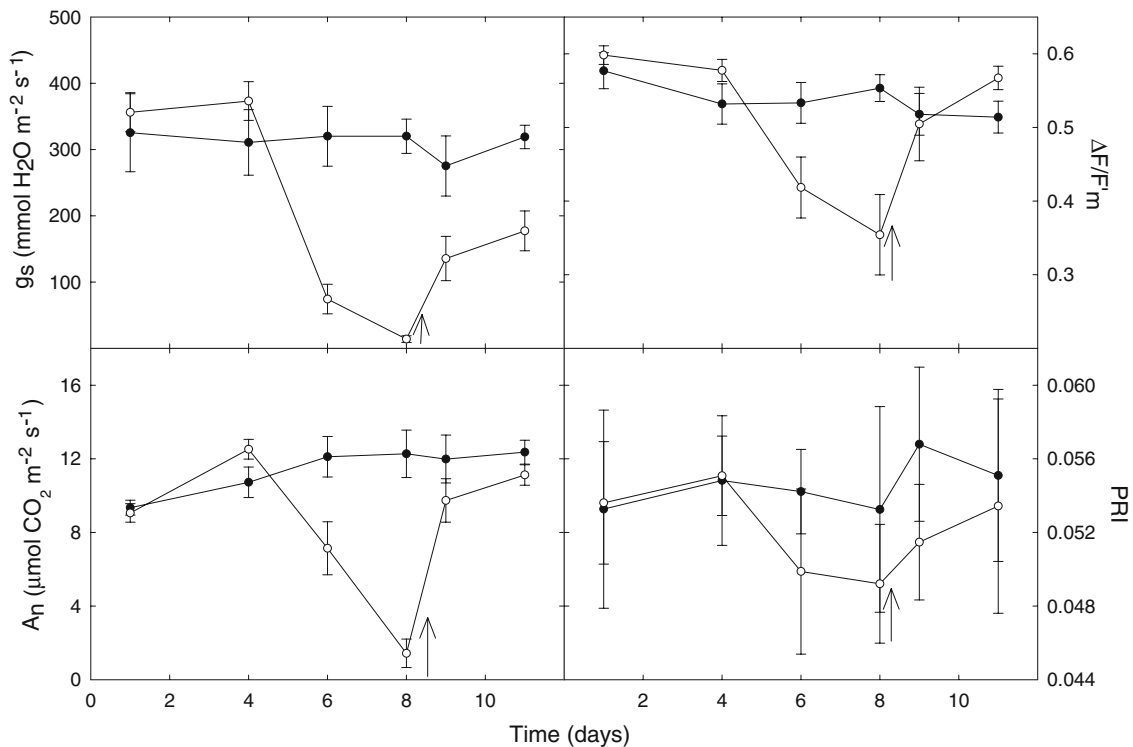


Fig. 6 Effect of experimental drought and re-watering on stomatal conductance (g_s), net photosynthesis (A_n), light-adapted fluorescence ($\Delta F/F'_m$), and PRI over time Arrows indicate when plants were re-

watered. Closed and open symbols represent mean ± 1 standard error for control and treatment plants, respectively

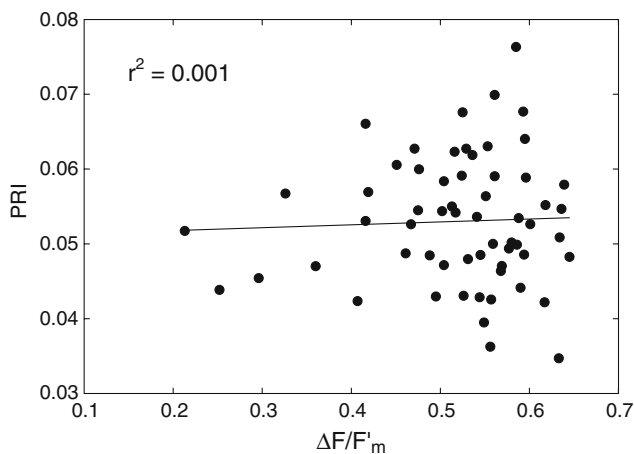


Fig. 7 Relationship between leaf-level PRI and $\Delta F/F'_m$ for drought-treated plants

E. umbellata was able to recover from drought treatment quickly and maintain high levels of photosynthesis after re-hydration. Relative to nearby native plants, *E. umbellata* was able to maintain a higher rate of photosynthesis with higher xylem pressure potentials. PRI was not effective at tracking changes in $\Delta F/F'_m$ during water stress in both field and laboratory studies. Future studies are needed to determine if trichome density changes throughout the summer, and if this has an effect on PRI as suggested by

Levizou et al. (2005). However, our results provide further evidence that *Elaeagnus* species use other energy dissipative mechanisms for protection of the photosystem. All of these physiological and morphological characteristics enhance the invasive success of *E. umbellata* and enable invasion of areas where water availability may limit other species.

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